UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

October 6, 2008

MEMORANDUM

Subject: Efficacy Review for Enviro San, EPA Reg. No. 1677-185; DP Barcode: D354861

From: Ibrahim Laniyan, Microbiologist
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10/15/08

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To: Marshall Swindell / Karen Leavy
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Ecolab Inc.
370 N. Wabasha Street
St. Paul, MN 55102

Formulation from the Label:

Active Ingredient	% by wt.
Hydrogen Peroxide	11.2 %
Peroxyacetic Acid	15.2 %
Inert Ingredients	73.6 %
Total	100.0 %

I. BACKGROUND

The product, Enviro San (EPA Reg. No. 1677-185), is an EPA-approved sterilant, sanitizer, sanitizing rinse, and antimicrobial rinse for use on hard, non-porous surfaces in commercial and food processing environments. The label claims that the product is effective as a sterilant in the presence of 500 ppm hard water. The applicant requested an amendment to the registration of this product to include three additional sterilant uses: (1) use of the product, Enviro San, with an adjuvant, ES-1000, at 50°C; (2) use of the product, Enviro San, alone at 50°C; and use of the product, Enviro San, alone at 60°C. Studies were conducted at Ecolab Inc., Ecolab Schuman Campus, located at 655 Lone Oak Drive, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated June 21, 2008), EPA Form 8570-35 (Data Matrix), three studies (MRID 474681-01 through -03), Statements of No Data Confidentiality Claims for all three studies, and the proposed label.

II. USE DIRECTIONS

The product is designed for sterilizing surfaces such as non-porous manufacturing, filling, and packaging materials and equipment for food processing. The proposed label indicates that the product may be used on surfaces such as aluminum, high density polyethylene (HDPE), polyethylene terephthalate (PET), and stainless steel. [The proposed label includes directions for use on non-porous surfaces only.] Directions on the proposed label provided the following information regarding preparation and use of the product as a sterilant: Remove gross soil particles from surfaces. Thoroughly clean surfaces and follow with a potable water rinse. Prepare a use solution by adding 5.0 ounces of the product and 0.2 ounces of the adjuvant (i.e., ES-1000 concentrate) to 1 gallon of water. Apply the use solution by immersion, coarse spray, or circulation techniques. Surfaces should be exposed to the use solution for a period of not less than 19 seconds at 60-70°C. After thorough draining, rinse surfaces with disinfected or sterile water.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sterilizers: The AOAC Sporicidal Test is recommended for substantiating sterilizing claims. The following information applies to all products represented as sporicidal or sterilizing agents. Sixty carriers, representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops), must be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 product samples representing 3 different product lots, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) that is recommended for use in a specific device must be tested using the AOAC Sporicidal Test in that specific device and according to the directions for use of that specific device. Killing on all of the 720 carriers is required; no failures are permitted. Data to support sterilizing claims must be confirmed by tests conducted by a second, independent laboratory of the applicant's choice (other than the laboratory that developed the original data). The following minimal confirmatory data must be developed on one sample of the product: Thirty carriers, representing each of the 2 types of surfaces (porcelain penicylinders and silk suture loops) against spores of both *Bacillus subtilis* and *Clostridium sporogenes* (a total of 120 carriers) by the AOAC Sporicidal Test. Killing on all of the 120 carriers is required; no failures are permitted.

Supplemental Claims: On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 474681-01 "Commercial Sterilant Efficacy of Enviro San with ES-1000 Adjuvant at 50°C," by Kris Owens. Study conducted at Ecolab Inc. Study completion date – May 20, 2008. Study Identification Number 0800005.

This study was conducted against *Bacillus subtilis* (ATCC 19659), *Bacillus cereus* (ATCC 14579), and *Clostridium sporogenes* (ATCC 3584). Three lots (Lot Nos. J082971, J102572, and J010981) of the product, Enviro San, combined with three lots (Lot Nos. DJR108A, DJR108B, and DJR108C), of the adjuvant, ES-1000, were tested. The study referenced the EPA Pesticide Assessment Guidelines, Subdivision G, 91-2(a), modified for commercial sterilant testing. One product and adjuvant combination (i.e., Product Lot No. J082971 with Adjuvant Lot No. DJR108A) was at least 60 days old at the time of testing. Use solutions were prepared by adding ~24 g of the product and 1.18 g of the adjuvant to ~1175 g of 500 ppm AOAC synthetic hard water (titrated at 490-500 ppm). Sixty (60) polished stainless steel penicillin cup carriers were immersed in a 6-14-day old test spore suspension for 15-25 minutes at room temperature, at a ratio of 1 carrier per 1 mL of suspension. The carriers were then dried in a vacuum dessicator for at least 24 hours prior to use. Each carrier was transferred to 10 mL of the use solution for a contact time of 40 seconds at 50±2°C. Following exposure, each carrier was transferred to individual tubes of Fluid Thioglycollate with 0.5% sodium thiosulfate. Each carrier was then transferred to a secondary subculture tube of Fluid Thioglycollate with 0.5% sodium thiosulfate. Subculture tubes were incubated for 21 days at 35±2°C. Following incubation, the subculture tubes were visually examined for growth. Tubes demonstrating no growth were heat-shocked for 20 minutes at 80±2°C and re-incubated for 72±4 hours at 35±2°C. Controls included those for purity, sterility, viability (i.e., positive control), enumeration of the test systems, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided data for a failed chemical quality verification set up on March 24, 2008. In that testing, the response factor for the standards failed; therefore, analysis of the method assurance standards and of the samples was terminated. Thus, the test was invalid. The use solution was prepared again and testing was repeated on April 2, 2008. See Appendix B of the laboratory study.

2. MRID 474681-02 "Commercial Sterilant Efficacy of Enviro San at 50°C," by Kris Owens. Study conducted at Ecolab Inc. Study completion date – May 20, 2008. Study Identification Number 0800006.

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). Three lots (Lot Nos. J082971, J102572, and J010981) of the

product, Enviro San, were tested. The study referenced the EPA Pesticide Assessment Guidelines, Subdivision G, Section 91-2(a), modified for commercial sterilant testing. At least one of the product lots tested (i.e., Lot No. J082971) was at least 60 days old at the time of testing. Use solutions were prepared by adding ~20 g of the product and ~980 g of 500 ppm AOAC synthetic hard water (titrated at 490-510 ppm). Sixty (60) polished stainless steel penicillin cup carriers were immersed in an 8-day old test spore suspension for 15-25 minutes at room temperature, at a ratio of 1 carrier per 1 mL of suspension. The carriers were then dried in a vacuum dessicator for at least 24 hours prior to use. Each carrier was transferred to 10 mL of the use solution for a contact time of 40 seconds at 50±2°C. Following exposure, each carrier was transferred to individual tubes of Fluid Thioglycollate with 0.5% sodium thiosulfate. Each carrier was then transferred to a secondary subculture tube of Fluid Thioglycollate with 0.5% sodium thiosulfate. Subculture tubes were incubated for 21 days at 35±2°C. Following incubation, the subculture tubes were visually examined for growth. Tubes demonstrating no growth were heat-shocked for 20 minutes at 80±2°C and re-incubated for 72±4 hours at 35±2°C. Controls included those for purity, sterility, viability (i.e., positive control), enumeration of the test systems, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

3. MRID 474681-03 "Commercial Sterilant Efficacy of Enviro San at 60°C," by Kris Owens. Study conducted at Ecolab Inc. Study completion date – May 2, 2008. Study Identification Number 0800007.

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). Three lots (Lot Nos. J082971, J102572, and J010981) of the product, Enviro San, were tested. The study referenced the EPA Pesticide Assessment Guidelines, Subdivision G, Section 91-2(a), modified for commercial sterilant testing. At least one of the product lots tested (i.e., Lot No. J082971) was at least 60 days old at the time of testing. Use solutions were prepared by adding ~27 g of the product and ~973 g of 500 ppm AOAC synthetic hard water (titrated at 490-500 ppm). Sixty (60) polished stainless steel penicillin cup carriers were immersed in an 8-day old test spore suspension for 15-25 minutes at room temperature, at a ratio of 1 carrier per 1 mL of suspension. The carriers were then dried in a vacuum dessicator for at least 24 hours prior to use. Each carrier was transferred to 10 mL of the use solution for a contact time of 19 seconds at 60±2°C. Following exposure, each carrier was transferred to individual tubes of Fluid Thioglycollate with 0.5% sodium thiosulfate. Each carrier was then transferred to a secondary subculture tube of Fluid Thioglycollate with 0.5% sodium thiosulfate. Presumably, subcultures were incubated and heat-shocked according to the testing protocol. [The laboratory failed to include a complete discussion of the efficacy test procedure on page 11 of the laboratory study. For the two other efficacy studies provided in this data package, the subculture tubes were incubated for 21 days at 35±2°C. Following incubation, the subculture tubes were visually examined for growth. Tubes demonstrating no growth were heat-shocked for 20 minutes at 80±2°C and re-incubated for 72±4 hours at 35±2°C.] Controls included those for purity, sterility, viability (i.e., positive control), enumeration of the test systems, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
		Lot No. J082971 + DJR108A	Lot No. J102572 + DJR108B	Lot No. J010981 + DJR108C	
474681-01	*Clostridium sporogenes*	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	4.6×10^5
	Bacillus subtilis	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	7.6×10^5
	Bacillus cereus	1° = 0/60* 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	7.7×10^5
		Lot No. J082971	**Lot No. J102572**	**Lot No. J010981**	
474681-02	*Clostridium sporogenes*	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	3.4×10^5
	Bacillus subtilis	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	5.4×10^5
474681-03	*Clostridium sporogenes*	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	4.3×10^5
	Bacillus subtilis	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	1° = 0/60 2° = 0/60	6.7×10^5

* Tube #9 was positive for growth (pre-heat shock). The laboratory study stated that "[during testing, the carrier made contact with the outside of the second subculture tube. The attempt to subculture yielded no growth indicating that the organism was not viable. A phase contrast microscope showed cocci cells in chains, indicating that the organism was a contaminant and not the test system."

VI. CONCLUSIONS

1. The efficacy data provided for three additional sterilant uses of the product (MRID 474681-01 through 474681-03) **support** the use of the product, Enviro San, as a sterilant against the following microorganisms on hard, non-porous surfaces in the presence of 500 ppm hard water for the listed conditions:

Bacillus cereus	40 seconds; 50°C; product plus adjuvant
Bacillus subtilis	40 seconds; 50°C; product plus adjuvant
Clostridium sporogenes	40 seconds; 50°C; product plus adjuvant
Bacillus subtilis	40 seconds; 50°C; product only
Clostridium sporogenes	40 seconds; 50°C; product only
Bacillus subtilis	19 seconds; 60°C; product only
Clostridium sporogenes	19 seconds; 60°C; product only

Efficacy data provided for the three additional sterilant uses of the product demonstrated complete killing in the subcultures of the required number of carriers (i.e., 360 hard, non-porous carriers) tested against the required number of product lots (i.e., 3). Neutralization confirmation testing showed positive growth of the microorganisms. The viability controls were positive for growth. Purity controls were reported as pure. The sterility controls did not show growth. Test spores showed resistance to acid for at least 2 minutes.

VII. RECOMMENDATIONS

1. The proposed label does not include directions or claims of effectiveness for the three additional sterilant uses:

(1) **40 seconds contact time** for use of the product, Enviro San, with an adjuvant, ES-1000, at 50°C;

(2) **40 seconds contact time** for use of the product use of the product, Enviro San, alone at 50°C;

(3) **19 seconds contact time** for use of the product use of the product, Enviro San, alone at 60°C.

The efficacy data provided **support** the three additional sterilant uses of the product.

2. The applicant must make the following changes to improve the proposed label:

- Under the "Environmental Hazards" section of the proposed label, change "permitting authority" to read "**the** permitting authority."

- In the first paragraph on page 3 of the proposed label, change "equipment dairies" to read "equipment **in** dairies."

- Under the "Commercial Sterilization" section of the proposed label, as appropriate, include a statement similar to the following: "When sterilizing complex equipment such as [identify the type of equipment intended for treatment, in addition to any description of surface composition]: Fully disassemble the equipment according to the manufacturer's instructions; clean and rinse all hollow areas making sure that the equipment is completely free of all soap before immersion in the use solution); sterilize the equipment according to product label directions making sure that all hollow areas are filled with the use solution; avoid entrapment of air bubbles); and completely reassemble under aseptic conditions.

- Under the "Sanitizing Food Contact Surfaces" section and the "Sanitizing Non-Food Contact Surfaces" section of the proposed label, change "by governing sanitary code" to read "by **the** governing sanitary code."

- Under the "Sanitizing Non-Food Contact Surfaces" section of the proposed label, change "to **sanitizer** the surfaces" to read "to **sanitize** the surfaces."

- Under the "Pesticide Storage" section of the proposed label, add instructions that specify what to do if the product or use solution leaks or spills from its container.

- Under the "Container Disposal" section of the proposed label, change "or incineration" to read "or **by** incineration."